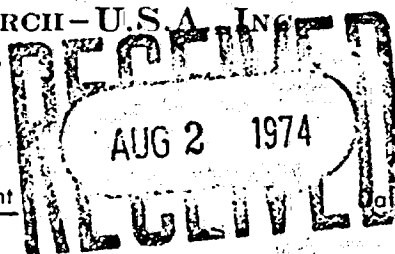


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THE COUNCIL FOR TOBACCO RESEARCH - U.S.A. INC.

110 EAST 50TH STREET  
NEW YORK, N. Y. 10022  
(212) 421-8885

Application for Research Grant  
(Use extra pages as needed)



Date: 7/25/74

1. Principal Investigator ( give title and degrees ):

Anthony A. Albanese, Ph.D., Director of Nutrition and  
Metabolic Research Division

2. Institution and address:

Burke Rehabilitation Center  
White Plains, New York 10605

3. Department where research will be done or collaboration provided:

Nutrition and Metabolic  
Research Division

4. Short title of study:

"Effect of Cigarette Smoking on Utilization and Metabolism of  
Ascorbic Acid in Man".

5. Proposed starting date:

September 1, 1974

6. Estimated time to complete:

1 year

7. Brief description of specific research aims:

In 1961, Larson, Haag and Silvette raised questions concerning the effect of smoking on the availability of Vitamin C in man ( 1 ). Since then a number of reports have appeared which imply that there may be an untoward metabolic relationship in man between cigarette smoking and ascorbic acid levels in the urine, plasma and white blood cells ( 2 ). A careful review of this literature reveals that many of the confusing deductions from these studies arise primarily from choice of analytical methods and/or body fluids examined. Currently leucocyte determinations

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are widely regarded as the more reliable measure of vitamin C nutrition in man. However, the broad range of "normal values" reported by various investigators prompted us to examine the available methods for possible experimental errors. This search disclosed that the prevailing practice of reporting ascorbic acid content in terms of  $10^8$  white blood cells constitutes a major source of experimental error. To overcome this and other problems a new procedure has been developed and thoroughly tested for accuracy during the past 2 years. In our method ascorbic acid content is measured in terms of leucocyte protein, rather than a leucocyte count.

The main objective of the present proposal is to investigate the effects of cigarette smoking on vitamin C by our method rather than an arbitrary value of  $10^8$  cells. It is felt that this chemically definitive approach to the problem should resolve and clarify many of the existing ambiguities regarding the relationship between smoking and vitamin C metabolism.

Significance of Research Aims: In recent years the clinical interest in ascorbic acid nutrition has grown to public health proportions as a result of Linus Pauling's hypothesis that subnormal vitamin C nutrition may increase the incidence of upper respiratory infections ( 3 ). This concept which has been highly criticized is now finding significant support in the reports of Anderson and associates ( 4 ), Wilson and Loh ( 5 ) and more recently, by as yet, unpublished study of the USPHS.

Apart from these new clinical implications, ascorbic acid has long been known to be involved in a number of physiologically important processes ( 6 ). Namely, it is a prerequisite for the formation of collagen, intercellular cement, dentine, cartilage, callus, osteoid tissue of the bone, blood vessel walls and the connective tissue; and is indispensable in the healing of wounds and the union of fractures. Ascorbic acid is important in tooth development and is a factor in resistance to infection. It is actively involved in certain vital functions of the body, such as carbohydrate and protein metabolism, cellular oxidation and hydrogen transfer. In its basic role, that of activator of tissue growth, its chief metabolic functions are thought to concern the adrenal cortex, pituitary gland, corpus luteum, brain, pancreas, liver and spleen, in which organs it is found in highest concentration. Ascorbic acid is also a strong reducing agent and an effective antioxidant.

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8. Brief statement of working hypothesis:

It has now been established beyond a reasonable doubt that qualitative or quantitative colorimetric test on urine, plasma or other body fluids are of limited, if not misleading, value for evaluation of vitamin C nutrition or detection of deficiencies in man ( 7,8 ). On the other hand, leucocyte vitamin C determinations are widely regarded as reliable and sensitive measures of ascorbic acid nutrition. However, in as yet unpublished studies we found that an accurate and clinically useful assessment of human vitamin C metabolism requires determination of both plasma and leucocyte protein ascorbic acid content. These investigations indicate plasma-leucocyte protein vitamin C ratios maybe the most significant metabolic parameter for evaluating the effects of endogenous and exogenous factors on the utilization and numerous biochemical functions of ascorbic acid. Accordingly, it is felt that a study of the effect of cigarette smoking on vitamin C in terms of changes of the plasma-leucocyte protein ascorbic acid ratios would provide data useful for clarification of existing controversies on this subject.

9. Details of experimental design and procedures ( append extra pages as necessary )

Protocol: Measurements of ascorbic acid will be done on blood samples collected 2 hours after a sample breakfast of toast and coffee ( 200 calories ) of 50 healthy normal male and female smokers of 1 pack or more of cigarettes and 50 non-smokers in the age range of 16 to 50 years. Details of duration and extent of smoking habits elicited by a questionnaire will be correlated with the biochemical findings. The subjects selected for these studies will be paid volunteers who have signed an informed consent waiver. They will be hospital personnel who have passed the requirements of the annual employee health program.

Methods: Ascorbic acid levels in the leucocytes and plasma will be determined colorimetrically by the 2-4 dinitrophenyl hydrazine reaction. Ascorbic acid content of the leucocytes will be measured in terms of micrograms per  $10^8$  leucocyte as described by Denson and Bowers ( 9 ) and per milligrams of leucocyte protein by the method developed in our laboratory ( 10 ). It is expected that this dual analytical approach will serve to clarify such relationships as may exist between cigarette smoking and vitamin C nutrition in man.

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## REFERENCES

1. Larson, P.S., Haag, H.B. and Silvette, H. Clin. Pharm. and Therapeutics 2, 80 1961
2. Pelletier, O. Nutrition Today 12-15 Autumn 1970
3. Pauling, L. Vitamin C and the Common Cold, San Francisco 1970; also Proc. Nat. Acad. Sci. 67, 1643 1970
4. Anderson, T.W., Reid, D.B.W. and Beaton, B.H. Canadian Med. Assoc. J. 107, 503 1972
5. Wilson, C.W. and Loh, H.S. Lancet, March 24, 1972
6. Goodhart, R.S. and Shils, M.E. Modern Nutrition in Health and Disease, Lea Febiger, Phil. 1973 pp. 244-255
7. Pelletier, O. Am. J. Clin. Nutr. 21, 1259 1968; 23, 520 1970
8. Calder, J.H., Curtis, R.C. and Fore, H. Lancet March 9, 1963 556
9. Denson, K.W. and Bowers, E.E. Clinical Science 21, 157 1961
10. Albanese, A.A. in press.

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10. Physical Facilities Available:

Nutrition and Metabolic Research Division previously visited and known to Dr. R. C. Hockett and Dr. McKean Cattell; and more recently Dr. Fredrick W. Nordsiek.

11. Additional Requirements:

None anticipated. Instrumentation required for the conduct of the described studies is on hand.

12. Biographical Sketches of All Principal and Professional Personnel:

See attached sheets.

13. PUBLICATIONS

Of a total of 229 publications, 77 have appeared in this decade and five invited scientific exhibits at national and international meetings.

Reviews and chapters in our area of competence have been prepared by invitation in the following text and reference books.

1. Albanese, A. A. General Nutrition of the Aging Patient in "Clinical Principles and Drugs in the Aging" (J. T. Freeman, editor), C. C. Thomas, Springfield, 1963, pp. 173-218.

2. Albanese, A. A. and Orto, L. A. The Proteins and Amino Acids in "Modern Nutrition in Health and Disease" (M. Wohl and R. Goodhart, editors), Lea and Febiger, Philadelphia, 3rd edition 1964; 4th edition 1968; 5th edition 1973

3. Albanese, A. A. Human Metabolism of Amino Acids and Fats in "The Art of Predictive Medicine" (W.L. Marxer and G. R. Cowgill, editors), C. C. Thomas, Springfield, 1967, pp. 99-104.

4. Albanese, A. A. Clinical Techniques for Evaluating Anabolic Agents in "Animal and Clinical Pharmacologic Techniques in Drug Evaluation" (P.E. Siegler and J.H. Mayer III, editors), Volume 2, Year Book Medical Publishers, Chicago, 1967, pp.763-778.

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5. Albanese, A. A. and Orto, L. A. Urinary Excretion of Amino Acids in "Newer Methods of Nutritional Biochemistry" (A. A. Albanese, editor) Volume III, Academic Press, New York, 1967, pp. 1-100.
6. Albanese, A. A. Nutritional and Metabolic Effects of Some Corticosteroids in Man in "Circadian Rhythms and New Aspects of Corticosteroids."

Research Reports 1970-1972

1. Albanese, A.A., Orto, L.A., Zavattaro, D.N., and De Carlo, R. Protein Metabolic Significance of Blood Ribonuclease Levels in Man. Nutr. Repts. International 4, 151 (1971).
2. Albanese, A. A., Orto, L.A., and Zavattaro, D.N. Nutritional and Metabolic Effects of Physical Exercise. Nutr. Repts. International 3, 165 (1971).
3. Albanese, A.A., Orto, L.A., Wein, E.H., and Zavattaro, D.N. Effect of Cigarette Smoking on Protein and Amino Acid Metabolism. I. Tryptophan. Nutr. Repts. International 5, 245 (1972).
4. Albanese, A.A., Lorenze, E.J., Orto, L.A., Wein, E.H., Zavattaro, D.N., and De Carlo, R. Nutritional and Metabolic Effects of Some Newer Steroids. VI. Serum Ribonuclease. N. Y. State J. Med. 72, 1595 (1972)
5. Albanese, A.A., Edelson, A.H., Woodhull, M.L., Lorenze, E.J., Wein, E.H. and Orto, L.A. Effect of Calcium Supplement on Serum Cholesterol, Calcium, Phosphorus and Bone Density of "Normal Healthy" Elderly Females. Nutr. Repts. International 8, 119 (1973)

editors, G. G. and J. H. Smith, 1973, pp. 1-100.

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14. Budget:

A. Salaries ( Personnel by names ) % Time Amount

Professional

Anthony A. Albanese, Ph.D. 10% 2,400

Technical

Evelyn H. Gruber,

Chemical Technologist 100 13,500

Secretarial services 20 2,000

Sub-Total \$17,900

B. Consumable Supplies ( list by categories )

Reagents \$600 \$950 950

Glassware 350

Sub-Total \$18,850

C. Other Expenses ( itemize )

Publication costs \$600 \$2,200

Travel to meetings 600

Fees for test subjects 1,000

Sub-Total \$21,050

D. Permanent Equipment ( itemize )

None \$21,050

E. Indirect costs ( 15% of A+B+C )

\$3,200 3,200

TOTAL \$24,250

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Ascorbic Acid Needs of the Elderly	Private Foundation	\$8,960	July 1, 1974 June 30, 1975

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None	None		

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Anthony A. Albanese, Ph.D.

Signature Anthony A. Albanese Date 7/25/74

Telephone 914-948-3676  
Area Code Number Extension

Checks payable to

Curtis Rehabilitation Hospital

Mailing address for checks

White Plains New York

Responsible officer of institution

Typed Name Fletcher H. McDowell

Title Medical Director

Signature Fletcher H. McDowell Date 7/24/74

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